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ALLEVIATION OF SALT-INDUCED SEED DORMANCY IN THE PERENNIAL HALOPHYTE *CRITHMUM MARITIMUM* L. (APIACEAE)

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Abstract

Crithmum maritimum L. (Apiaceae) is a perennial local oilseed halophyte. For a better understanding of the eco-physiology of salt tolerance at the germinative stage of this species, we investigate here the effects of nitrate, thiourea, and priming on its seed germination under saline conditions. Germination was strongly inhibited by increasing salinity. While nitrate supply was effective in alleviating salt-induced seed dormancy under both non- and saline and conditions, thiourea improved germination only at moderate salt concentrations. Priming with both water and NaCl accelerated the germination process on salt free medium, whereas PEG 6000 delayed it in distilled water. The different priming agents used in the present work impaired both germination capacity and germination velocity index at 100 mM NaCl, but seeds remained viable since being able to germinate after their transfer to distilled water.
Keywords: germination, halophyte, nitrate, priming, salinity, thiourea

Introduction

Germination is one of the most salt-sensitive plant growth stages and severely inhibited with increasing salinity both in glycophytes and halophytes (Sosa *et al*., 2005). However, nitrogenous compounds, such as nitrate and thiourea, are known to promote seed germination under salinity (Egley, 1995). Nitrogenous compounds were also effective in alleviating salt-induced dormancy in several halophytes (Khan *et al*., 2003). Salt-induced seed dormancy can be broken by seed priming. Seed priming is soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radicle emergence (McDonald, 1999). Seed priming accelerates seed germination and seedling establishment under both normal and stressful environments (Ashraf & Foolad, 2005). It has been well documented that seed priming causes metabolic changes in germinating seed physiology, e.g., cell cycle related event (De Castro *et al*., 2000), endosperm weakening by hydrolase activities (Groot *et al*., 1988; Bradford *et al*., 2000), and mobilization of storage proteins (Job *et al*., 1997; 2000). However, positive effects of seed priming on seed germination depends on type of priming agent, duration of seed soaking, concentration priming agent (Ashraf & Foolad, 2005). Polyethylene glycol (PEG) and NaCl are among the most used priming agents.

Crithmum maritimum L. (*Apiaceae*) (sea fennel or rock samphire) is a local oilseed halophyte occurring in rocky coasts. Its leaves are used as antiscorbatic, tonic, diuretic,

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and vermifuge (Ruberto *et al*., 2000). The present work is a part of a national program supported by the Tunisian Ministry of Scientific Research and Competence Development, aiming at the identification of local halophytes with ecological and/or economical potential. Keeping this in mind, the present study was aimed to assess up to what extent nitrate and thiourea supplied through the rooting medium have ameliorative effect on adverse effects of salt stress on seed germination of *Crithmum maritimum* L. In addition, ameliorative effect of halo-priming, hydro-priming and osmo-priming on early growth of *Crithmum maritimum* L. was also assessed.

Materials and Methods

C. maritimum L. mature fruits were harvested from the northern Tunisian coasts and disinfected for 5 min using calcium hypochlorite (3.5 %). In a first experiment, seeds were germinated for 40 days in Petri dishes (four replicates of 25 seeds each) on filter paper moistened with NaCl solutions (0, 50, 100, 150, and 200 mM), supplied or not with nitrate (20 mM) and thiourea (10 mM). Germination was carried out at 18-23°C temperature and 16-8 h light-dark regime. Priming experiments consisted in pre-treating seeds for 7 days with either H_2O (control), NaCl (50 or 300 mM) or isotonic polyethylene glycol (PEG 6000) solutions (–0.22 MPa and -1.3 MPa). Seeds were then germinated for 40 days at 0 and 100 mM NaCl (4 replicates of 25 seeds each per treatment) under the same conditions above described.

Germination rate was calculated using a modified Timson's index of germination velocity (Σ G / t), where *G* is the percentage of seed germination at 2 day-interval and *t* is the total time of germination (Khan & Ungar, 1984). Recovery germination rate was calculated using the relation [(a-b) / (c-b)]*100, where *a* is the total number of seeds germinated after being transferred to distilled water, *b* is the total number of seeds germinated in saline solution, and *c* is the total number of seeds. Statistical analysis (one-way ANOVA) was performed using the statistical software SPSS 10.0.

Results

Salt concentrations exceeding 50 mM NaCl delayed and reduced seed germination (Fig. 1). However, thiourea applied through the rooting medium promoted seed germination, particularly at 100 mM NaCl. Likewise, total germination percentage and rate of germination were also enhanced due to thiourea applied in growth medium (Figs. 1A and 1B). Nitrate addition to the growth medium also improved the germination percentage (Fig. 1C) and speed of seed germination (Fig. 1D), particularly at 100-150 mM NaCl. However, addition of nitrate to the growth medium was more effective in improving both seed germination %age and speed of germination.

Imposition of salt stress reduced the seed germination and germination velocity index of both primed and non-primed seeds. However, this reducing effect was less in hydroprimed seeds. Seed soaking with 50 mM NaCl or PEG_{6000} slightly reduced the germination percentage under both non-stress and salt stress conditions (Fig. 2A; 2B). However, seed primed with 300 mM NaCl almost did not change seed germination and germination velocity index of both non-stressed and stressed plants (Fig 2A). It is interesting to note that seeds failed to germinate at 100 mM NaCl were transferred into distilled water, which exhibited higher germination %age but lower than primed seeds germinated in distilled water (85-95 %) (Fig. 3).

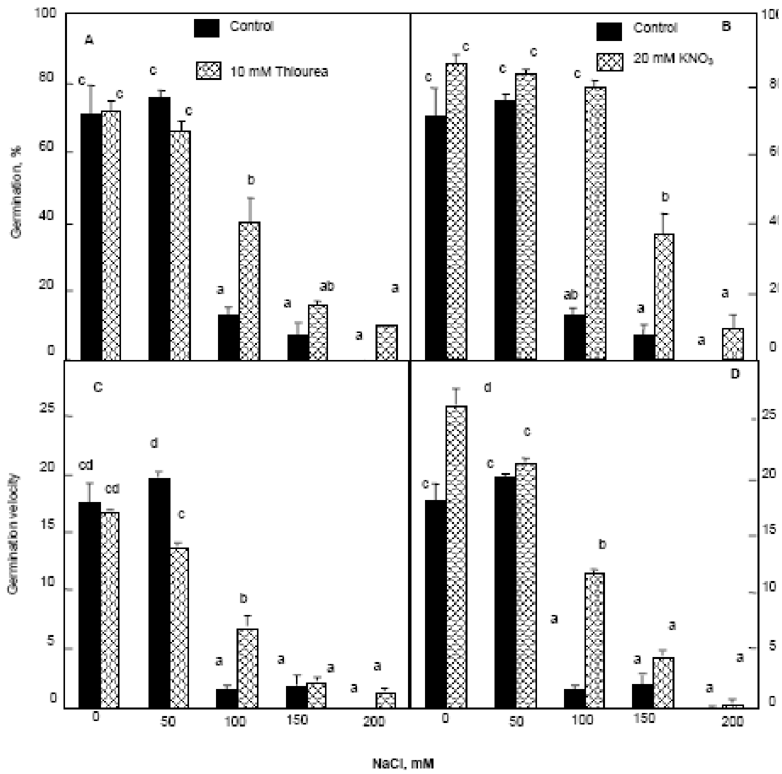

Fig. 1. Effect of thiourea (10 mM) and nitrate (20 mM) supply on seed germination percentage (A and C, respectively) and Timson's germination velocity index (B and D, respectively) of *C. maritimum* L. under saline conditions. Means (n = 4) with at least one same letter are not significantly different at P < 0.05, Tukey's test.

Discussion

Seed germination of *C. maritimum* L. was inhibited by salt concentrations exceeding 50 mM NaCl. Such a result is common among halophytes which show their highest germination in free- and low-salt media (Debez *et al*., 2004; Sosa *et al*., 2005). Although salinity may not alter seed viability, it could influence and/or block some physiological processes. The inhibitory effect of salinity on the rate and final germination percentage of *C. maritimum* L. was partially alleviated by the application of nitrate or thiourea. This result is in agreement with those observed in *Zygophyllum simplex* (Khan & Ungar, 1997). In *Allenrolfea occidentalis* (Gul & Weber, 1998) and *Atriplex prostrata* (Khan *et al*., 2003) these two nitrogen compounds completely alleviated the salt-induced dormancy. Nevertheless, in *C. maritimum* L., nitrate was more effective than thiourea. Similar results were obtained for *Allenrolfea occidentalis* seeds as germinated under high saline conditions 600 and 800 mM (Gul & Weber, 1998).

In salt-free medium, germination of *C. maritimum* L. seeds pre-treated with water (hydropriming) and NaCl (osmopriming) was accelerated. Such a positive effect is

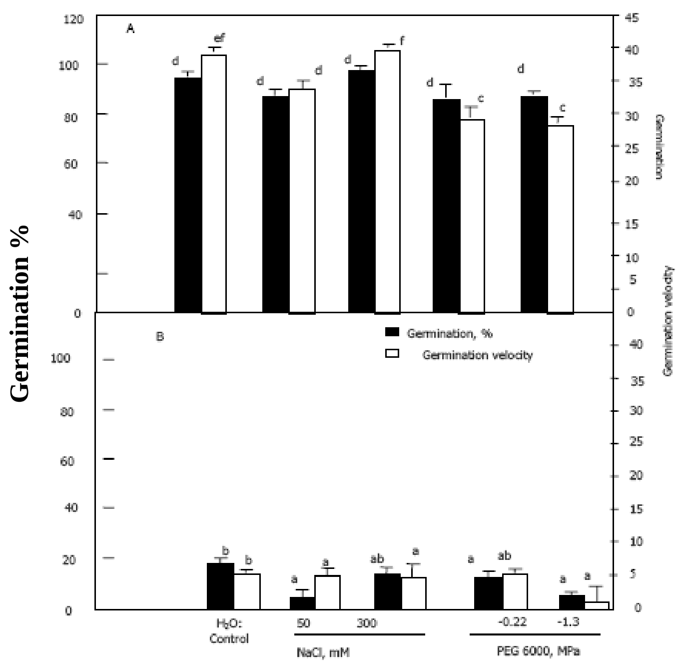

Fig. 2. Priming effect on *C. maritimum* L. seed germination. (A) germination percentage and Timson's germination velocity index of primed seeds after their transfer into distilled water for 40 days. (B) final germination percentage and Timson's germination velocity index of primed seeds after their transfer in 100 mM NaCl. Seeds were primed with H_2O (control), NaCl (50 mM and 300 mM) or iso-osmotic PEG 6000 solutions (-0.22 and -1.3 MPa, respectively). Means (n = 4) with at least one same letter are not significantly different at P < 0.05, Tukey's test.

corroborates previous reports on *Momordica charantia* (Chang & Sung, 2001), *Nyssa sylvatica* (Kmetz-Gonzalez & Struve, 2000), and *Lycopersicon esculentum* (Penaloza & Eira, 1993). Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells (McDonald, 1999). This action is important because it allows the subsequent development of the embryo, especially in seeds characterised by a morphological dormancy (immature embryo) like *Chamaecyparis nootkatensis* (Schimtz *et al*., 2001). In tomato, priming improved the germination capacity by increasing endosperm volume (Dahal *et al*., 1990). In the oilseed halophyte *C. maritimum* L., one could reasonably hypothesise that seed priming may (*i*) stimulate the metabolic reactions leading to the mobilisation of reserve lipids to initiate germination and (*ii*) to contribute to the integument contraction, thus facilitating the fast emergence of the radicle.

Salt-induced reduction in germination of primed seeds was not counteracted by seed priming, indicating that the beneficial effects of priming would be expressed only at moderate or low level of salt stress. Osmopriming for 7 days cause more adverse effects on seed germination and this may have been due to reduced uptake of mineral nutrients, or reduced synthesis or mobilisation food reserves (Ashraf & Foolad, 2005). The beneficial effect of halopriming as compared to osmopriming may have been due to contribution of Na^+ in osmotic adjustment and thus imbibitions. While working with

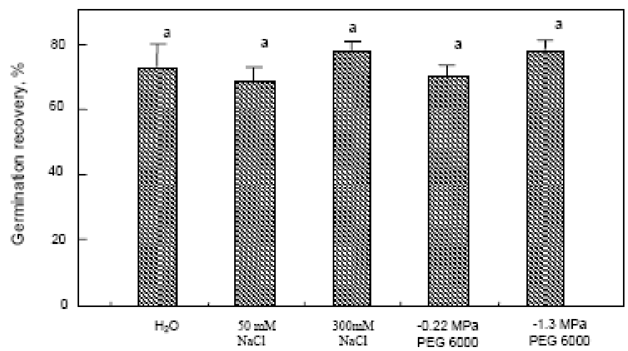

Fig. 3. Variation of the germination recovery according to the type of priming. Means (n = 4) at least one same letter are not significantly different at $P < 0.05$, Tukey's test.

Glycine max Khajeh-Hosseini *et al*. (2003) showed that seed germination was more reduced in PEG-induced osmotic stress than in iso-osmotic NaCl solution. Thus, beneficial effect of halopriming may have been due to its more contribution in osmotic adjustment.

Reversibility of salt-induced germination inhibition in *C. maritimum* L. indicates that salinity may inhibit the germination mostly via an osmotic effect preventing the imbibition phase to occur. Despite inter-and/or intra-specific variability in salt response at germination stage, the ability to conserve seed viability is common in halophytes which maintain seed banks able to germinate under favourable environmental conditions (Debez *et al*., 2004). Such a feature is of vital importance with respect to the ecology of halophytes, which grow on habitats characterised by great fluctuations in their salinity levels. Since hydropriming was more effective in alleviating adverse effects of salt stress, salt-induced osmotic stress was a dominant factor reducing seed germination. Furthermore, nitrate and thiourea improved germination indicating nitrogenous compounds are one of possible regulators of seed germination in soil (Egley, 1995).

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